Center for Produce Safety STEC Seasonality Project:
Romaine Lettuce Seasonal Risk in the California Central Coast Region
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CPS STEC Issue Brief 5: Bioaerosol Risk and Crop Setback Distances

Executive Statement: Dust dispersal from AFOs, CAFOs, manure stockpiles, composting facilities, cattle rangeland and pastures, hobby animal corrals, and during field-side storage and spreading of biological soil amendments of animal origin (BSAAO) represents a well-understood but substantially under-characterized risk. This risk must be considered in any dialogue regarding the inadequacy or overly protective metrics for setback distances from leafy greens production. Risk management decisions require information involving the specific conditions of each matrix of crop type, adjacent land and/or nearby land in combination with environment, weather, and crop management practices. This information integration is anything but simple for determining a root cause contribution for bioaerosols in outbreak investigations and developing effective preventive controls.

In simple terms, bioaerosols are small particulates of both organic and inorganic materials suspended in the air. The dispersal range is variable, depending on buoyancy traits, wind speed, and several other characteristics. Deposition of large particulates is often of limited range (<400 feet) unless the conditions involve high wind speeds, typically greater than 20 mph. However, long-distance and very-long-distance transport (>5,000 miles) has been demonstrated with plant colonizing bacteria, though not for foodborne pathogens in the context of fresh produce risk. A clear distinction for this possibility is that plant pathogens, scoured from the global jet stream by rain, have the potential for growth once deposited on a plant host; however, this has not been demonstrated for human foodborne pathogens. Bioaerosol risk assessment research for pathogens such as *E. coli* O157:H7 and *Salmonella* is far more limited than with environmental sources of respiratory pathogens and plant pathogens. As with plant pathogen transport, there are many models for aerosol risk assessments, which focus on spray dispersal and deposition distances of aqueous droplets (such as wastewater and manure lagoon effluent) and pathogen survival, but very few studies that support quantitative microbial risk assessment (QMRA) models to support sensible and predictive setbacks and adjacent land activity risk management policies.

Bioaerosol transport from presumptive regional root-source hosts, cow-calf and stocker cattle on adjacent or nearby sites to romaine lettuce (See CPS STEC Issue Brief 3: Regional Cattle Management Profile), is a longstanding element of root cause analysis hypothesis generation and efforts to rule in or rule out contribution to specific rather than speculative contamination events. Currently, the available evidence makes this scale of bioaerosol transport (miles) less credible than that due to areas of animal congregation near production fields. This, as well, remains largely unsubstantiated in the context of this CPS STEC project. The complexity and spatial gradient design of research studies needed to adequately inform root cause investigations, commercial risk management decisions, and uniform or flexible setback standards is arguably prohibitive. On-farm deployable and cost-manageable risk monitoring kits under development and validation may provide a grower tool(s) for site and seasonal risk profile and risk exposure knowledge.

The field of bioaerosol research is very complex. Applying the methods available for data collection and predictive models to establish practical standards for economically defensible leafy greens setback standards and specific metrics is extraordinarily complicated. Currently, permission to conduct such studies is also very limited, but this appears to be expanding. After more than two years of fact and data assembly, the uncertainty around the role of bioaerosol dust deposition onto surface water or a crop as the root cause of *E. coli* O157:H7 outbreaks within the CA Central Coast still looms large in discussions of whether traditional research approaches are meaningful. What is clear, is that single-site, small-scale investigations with few timepoints and few samplers is not a sensible experimental design for CPS research awards. Large-scale, long-term local and regional research studies are needed to develop the type of models needed for improvements in science policy and meaningful industry guidance.

Purpose: This Issue Brief provides a concise overview of the approaches and methods, as well as opportunities and challenges, in conducting open environment risk assessment research focused on bioaerosols (dust) as a source of foodborne pathogens. It can be tempting to oversimplify complex topics such as bioaerosols and produce food safety. However, as one key purpose is to provide background and guidance to prioritization and review of relevant research proposals for funding, the topic is presented in some depth at a lay-technical level.

Background: Animal feeding and compost production facilities are known to be associated with the potential for transfer of microorganisms via particulate suspension and off-site transport of bioaerosols (aka fugitive dust). Numerous studies conducted by public and private research entities, including many large-scale studies by the US EPA, provide general data and expectations for distances of particulate transport. Some of this data has been applied to models for establishing point-source bioaerosol management requirements to mitigate "fugitive dust" impacts to water sources, adjacent land use activities, and animal or human inhalation respiratory risks. Due to the low prevalence of foodborne pathogens in source materials, and issues of sensitivities to unsubstantiated attribution, these studies are dominated by following the transport of more ubiquitous mold spores, nonpathogenic microbes, diverse chemicals, or microbial endotoxins. It is important for the industry to appreciate that the main consequence of this absence of targeted research is a large knowledge gap in predicting viable transport and post-deposition survival on surface water, equipment and packing/packaging surfaces, or crops.

Regardless, there is long-standing evidence that persistence of *E. coli* O157:H7 in dry particulates is possible, and has been related to extended exposures within a linked outbreak (Varma et al. 2003). In 2001, almost 50% of dust samples collected in a county fair building housing animals were positive for the defining clinical case *E. coli* O157:H7. The investigation identified that many cases were likely exposed after removal of the animals when the space was used for a large dance. Some cases were months later. Positive samples included sawdust and fine dusts in the building rafters and were identical by molecular tests to clinical specimens, using techniques available at the time. Sawdust specimens collected 42 weeks after the investigation, prior to deep cleaning and facility improvements, remained positive for the outbreak isolate.

Though not based on bioaerosol risk alone, current leafy greens metrics require that growers keep a 30, 400, 1200 ft or 1 mile setback distance (or more in some private audit standards) from known or presumptive aerosolized organic matter which may contain foodborne pathogens, among other related risk factors. The foundation assumptions and appropriateness of these distances, and the basis for case-by-case setback modifiers, have not been adequately tested in environments pertinent to production of diverse commodities in California. Most risk assessment studies on airborne transport of human pathogens have been related to sewage wastewater disposal and have been conducted outside of the U.S. This research, much of it conducted in the 1970s and early 1980s evaluated the presence and/or persistence of pathogens conveyed to crops by spray irrigation and irrigation aerosols of sewage effluent. These data are available in a number of older reviews but, in this Issue Brief, we have largely chosen to limit Resources to more recent studies. Additionally, similar studies of animal waste lagoon effluent spray-drift from large water cannons have been conducted in many parts of the U.S. In comparison, very few studies have addressed dry particulate dispersal and deposition on romaine and other leafy greens in relevant CA Central Coast conditions and locations, though multiple attempts have been made to initiate this research in a sustained manner.

Published research that provides some directional information and methodology is provided in Resources. A CPS funded study has been cited and used, in part, as the basis for extending setback distances from 400 to 1,200 ft from large animal feeding operations (Berry et al. 2015; LGMA August 2, 2021). In this study by Berry et al., the outermost downwind trap crop was 600 ft from UDSA-managed cattle corrals, a characterized feedlot with *E. coli* O157:H7; viable *E. coli* O157:H7 was recovered at this distance during periods of greater animal activity and drag scrape–management of feedlot surfaces, both generating dust plumes. Warm air temperatures (but lower than at other sampling dates with no detection at 600 ft), animal transfer within the facility, equipment activity, and mild wind conditions were noted as potential factors to detect *E. coli* at the 600 ft zone. Deposition of bioaerosols was greater at further rather than closer trap crop distances from the corrals, under these conditions.

Relevant to risk and root cause considerations addressed during this CPS STEC project, there are several studies that evaluate and model the three steps of concern for bioaerosol generation and deposition onto romaine lettuce as a function of setback distances:

1. Aerosolization
    - Non-wind lift – active force (equipment, large animal activity)
    - Turbulent wind lift – wind force surface erosion (feedlot surface, compost facility, pasture)

2. Suspension
    - Topography determined wind eddies
    - Dust devil thermals and seasonal thermal wind downward bursts
    - Coast to interior valley wind speed, wind run (duration) and flow (often daily >30 °F temperature differentials north to south)
3. Deposition
    - Particle structure and buoyancy
        - % Dry matter
        - Coarse particulates
        - Fine particulates
        - Ultrafine particulates
    - Land topography and wind breaks/diversions
    - Crop growth habit
        - Romaine – maturity, head openness
        - Baby spinach and baby romaine
    - Weather events

A recent study conducted on particulate dispersal during field applications of poultry manure (Kabelitz et al. 2021), determined that the risk from small, dry particles (<15 μm) was substantially reduced at 400 m/~1,300 ft (this study was not related to leafy greens). It is challenging to extrapolate from the diverse studies reviewed during this project (not cited in text but available in Resources). The specific situational conditions related to any outbreak-source implicated production site are not known. There are recent efforts and ongoing research specific to romaine lettuce and leafy greens to fill this knowledge gap, but details of results are not yet available. Addressing the specific pattern of seasonally associated outbreaks by root cause investigation and by a more broadly approached regional research agenda must be evaluated within the constraints of common vs. appropriate bioaerosol sampling techniques.

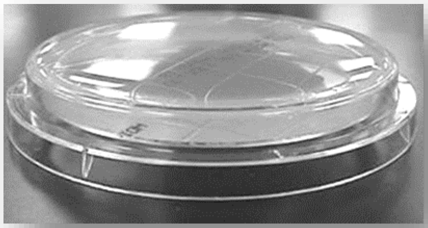
Overview of Some Sampling Techniques

The field of aerobiology and the techniques deployed to study bioaerosol generation (from a source), patterns of dispersal eddies and vectors, and deposition at the scale from leaf to farm to region or beyond, are complex. There is no single best approach or equipment, and many options exist, depending on the target (size, volume, natural or synthetic chemical, or biological). The proper selection of sampling equipment and method of detection for fecal indicators or pathogens is also determined by target and research objective.

The single biggest challenge to effectively addressing root cause questions on STEC outbreak seasonality is the deployment of bioaerosol capture devices in a sufficient array over a sufficient seasonal timeline. There are differences in conducting bioaerosol-focused root cause investigative studies adjacent to a large point-source (i.e., cattle CAFO, dairy, poultry houses, compost facility) compared with the CA Central Coast leafy greens production landscape/farmscape, which is typified more as a mosaic of non-point sources.

The majority of attempts to develop an actionable data set or predictive model are very limited in scope and therefore very difficult to apply, with confidence, to the establishment of setback parameters. Similarly, these limitations make the transition from hypothesis generation—around the role of bioaerosols from adjacent lands, to ruling in or ruling out this risk factor—fundamentally speculative within the CPS STEC Seasonality Project.

Passive bioaerosol sampling: Settling plates have long been a simple and comparatively inexpensive way to deploy a wide vector array of deposition sites to evaluate gradient effects of bioaerosol transport. The agar-only or nutrient-agar plates are left open for limited periods, primarily due to the potential for UV exposure and surface desiccation leading to loss of cell viability. There are several limitations to this approach, but the one most criticized is the passive nature of deposition onto the agar surface. Turbulent flow and boundary layer effects at surfaces can be physical barriers for the deposition to occur. Studies have measured and predicted the non-representative deposition of particulates in petri plate–based passive sampling due to the raised lip of the plate in horizontal and deflection events when mounted on an angled support facing into the direction of the wind stream. Small, convex agar-surface plates (i.e., Rodac™ plates; image to the left), typically used for surface contact-pressure sampling, reduce this boundary layer resistance to deposition and may be mounted and deployed as multi-plate arrays on a platform.



Various materials have been deployed as low-cost dust collection devices in environmental studies, adjacent to AFO and CAFO operations, and in recent on-farm studies within leafy greens production sites. Materials, such as cheesecloth, cellulose gel–coated cheesecloth, various microfiber swatches, and electrostatic-charged nanofiber “spider-web” strands in a wind-catcher frame, have been used in model systems and open-environment microparticle capture. Currently, data from these studies that would be most relevant to the focus of the CPS STEC Seasonality Project are not publicly available.

Andersen samplers: Andersen samplers are used to measure the concentration and particle or aggregate-size association of dust or bioaerosols. The sampler is a multi-orifice, cascade impactor designed to size-sort particles aerodynamically before they are collected on the surface of an agar medium. By understanding particle-size distribution from the types of sources mentioned above, it should be possible to predict their aerodynamics, and therefore distances to deposition, from well-established predictive models. The next data piece is concentration and viability by particle class size. These model-generated curves may allow the research community to test the current assumptions of food safety standards and metrics related to adjacent land use and proximity.

MAS air samplers: MAS air samplers are portable, lightweight samplers that can sample air at 100 L/min. Though particle-sizing data is not possible with these samplers, they are easy to use and program, give reliable and reproducible results, and are capable of collecting between 10 to 2000 liters from an airstream. Internally generated suction draws air into the sampler and onto agar plates or wetted filter paper placed inside the machine. Several studies have reported using this system with highly selective media to detect STEC, including *E. coli* O157:H7, in aerosolized dust from AFOs. The studies were successful in detecting *E. coli* indicators and pathogens by DNA molecular methods but were largely unsuccessful in recovery of colonies to confirm viability. Recovery of non-spore forming and stress-adapted bacteria associated with fine particulates can be improved by using multilayer wetted filter paper or soft-agar plates with pyruvic acid (for injured cell recovery), which have a higher water content and do not dehydrate with greater aerosol capture volumes. The basic non-selective capture surface system is also favorable for DNA extraction of captured bioaerosol dust for microbiome and metagenomic studies.

Glass impinger bioaerosol samplers: All glass impingers using a liquid-capture system (e.g., SKC BioSampler, right image) are considered the *de facto* reference standard for bioaerosol surveys and research. Collection directly into an osmotically balanced buffer minimizes cellular stress for maximum viable recovery of indicators or pathogens. Aerosol samples are pulled into the chamber at 12.5 L/min by three collection nozzles. Particulates are impinged on a water film developed on the sidewalls by a centrifugal-driven motion of the buffer solution created in the collection vessel during operation. Units can collect samples in up to 20 ml for recovery by plating on various resuscitation media or directly onto selective/differential agar media.

A significant limitation is having enough individual units, with associated vacuum sources, and power to deploy in a wide and deep vector array and all operating simultaneously under field conditions, as opposed to typical model wind tunnel studies. Depending on the bioaerosol type and source, these devices are prone to plugging with larger particulates or orifice binding, due to high humidity conditions. Even in an aqueous capture sampler, as is true for all bioaerosol sampling methods, risk assessment studies conducted without a low-nutrient, non-selective agar or membrane-based pre-selective growth resuscitation step are always subject to uncertainty in result interpretation when results are solely detection-negative.

Biosensors for On-Farm Bioaerosol Risk Assessments

Recognizing the practical challenges associated with bioaerosol research and the need for on-farm tools to assess site-specific risk, in 2018 CPS initiated a challenge award to technologists and researchers to develop simple, cost-effective, and rapid biosensor kits to detect the deposition of bioaerosols onto crops. The initial biosensor design was requested around indictors as a monitoring tool and to assist in developing a bioaerosol deposition "heat map" of a field or, potentially, field equipment. This heat map would be used to guide a deeper evaluation of pathogen risk exposure in areas determined to meet the biosensor detection threshold for the target fecal indicator or other biomarker. The absence of scientifically valid biomarker(s) in a well-saturated mapping scheme (the heat map) should support confidence in a positive harvest decision Several awards were made, and the further advancement of this technology and its practical application is the topic of ongoing CPS funded research (Verma, 2021). There are multiple modes of deployment and anticipated applications for on-farm biosensor concepts. If the final design and validation of an on-farm biomarker(s) tool is cost: benefit positive for growers and/or handlers, monitoring during seasonal crop development and incremental sampling may find broad adoption. Bioaerosol detection-mapping according to spatial proximity from domesticated animal facilities, wind direction and velocity variables, climatic/weather events, and other factors would be in reach for the industry but also likely to be a tool readily and affordably integrated into field and farm research projects and root cause investigations. With time and experience, a grower, or a handler, may have the flexibility to plant a crop, such as Romaine lettuce, closer to a domesticated animal production facility, for example, than currently allowed by industry setback standards. Proximity to a point source of potential pathogen contaminated bioaerosols will dictate the number and spatial deployment of the biosensor kit in concert with cropping practices and crop maturity.

Resources

Acosta-Martinez V, Van Pelt S, Moore-Kucera J, Baddock MC, Zobeck TM. 2015. **Microbiology of wind-eroded sediments: Current knowledge and future research directions**. Aeolian Res.18:99-113. https://doi.org/10.1016/j.aeolia.2015.06.001

Berry ED, Wells JE, Bono JL, Woodbury BL, Kalchayanand N, Norman KN, Suslow TV, Lopez-Velasco G, Millner PD. 2015. **Effect of proximity to a cattle feedlot on Escherichia coli O157:H7 contamination of leafy greens and evaluation of the potential for airborne transmission**. Appl Environ Microbiol. 81:1101-1110.

Brandi G, Sisti M, Amagliani G. 2000. **Evaluation of the environmental impact of microbial aerosols generated by wastewater treatment plants utilizing different aeration systems**. J Appl Microbiol. 88(5):845-852. doi: 10.1046/j.1365-2672.2000.01024.x.

Clauss M. 2020. **Emission of bioaerosols from livestock facilities - Methods and results from available bioaerosol investigations in and around agricultural livestock farming**, Thünen Working Paper 138a. 10.3220/WP1578391778000.

Douglas P, Robertson S, Gay R, Hansell AL, Gant TW. 2018. **A systematic review of the public health risks of bioaerosols from intensive farming.** Int J Hyg Environ Health. 221(2):134-173. doi: 10.1016/j.ijheh.2017.10.019.

Franchitti E, Pascale E, Fea E, Anedda E, Traversi D. 2020. **Methods for bioaerosol characterization: Limits and perspectives for human health risk assessment in organic waste treatment**. *Atmosphere*. 11:452. https://doi.org/10.3390/atmos11050452

Gediminas M. 2020. **Bioaerosol sampling: Classical approaches, advances, and perspectives**. Aerosol Sci Technol. 54(5):496-519. DOI: 10.1080/02786826.2019.1671950

Kabelitz T, Biniasch O, Ammon C, Nübel U, Thiel N, Janke D, et al. 2021. **Particulate matter emissions during field application of poultry manure – the influence of moisture content and treatment.** Sci Total Environ. 780:146652. 10.1016/j.scitotenv.2021.146652

Mahaffee WF, Stoll R. 2016. **The ebb and flow of airborne pathogens: monitoring and use in disease management decisions.** *Phytopathology* 106 (5):420–431. doi: 10.1094/PHYTO-02-16-0060-RVW.

Nag R, Monahan C, Whyte P, Markey BK, Flaherty VO, et al. 2021. **Risk assessment of Escherichia coli in bioaerosols generated following land application of farmyard slurry**. Sci Total Environ. 791:148189. https://doi.org/10.1016/j.scitotenv.2021.148189

Nag R, et al., 2022. **Quantitative microbial risk assessment associated with ready-to-eat salads following the application of farmyard manure and slurry or anaerobic digestate to arable lands**. Sci Total Environ. 806:151227. https://doi.org/10.1016/j.scitotenv.2021.151227

Preece S, Maghirang R, Amosson S, Auvermann BW. 2019. Dust Emissions from Cattle Feeding Operations. Texas A&M AgriLife Extension Service. AgriLifeExtension.tamu.edu

Purdy C, Nolan Clark R, Straus DC. 2007. **Analysis of aerosolized particulates of feedyards located in the Southern High Plains of Texas**. Aerosol Science and Technology, 41(5):497-509. DOI: 10.1080/02786820701225838

Ravva SV, Sarreal CZ, Mandrell RE. 2011. **Bacterial communities in aerosols and manure samples from two different dairies in Central and Sonoma Valleys of California**. PLoS ONE 6(2): e17281. https://doi.org/10.1371/journal.pone.0017281

Robertson S, Douglas P, Jarvis D, Marczylo E. 2019. **Bioaerosol exposure from composting facilities and health outcomes in workers and in the community: A systematic review update**. Int J Hyg Environ Health. 222(3):364-386. doi: 10.1016/j.ijheh.2019.02.006.

Santarpia J, Ratnesar-Shumate S, Haddrell A. 2020. **Laboratory study of bioaerosols: Traditional test systems, modern approaches, and environmental control.** Aerosol Science and Technology, 54(5):585-600. DOI: 10.1080/02786826.2019.1696452

Šantl-Temkiv T, et al. 2020. **Bioaerosol field measurements: Challenges and perspectives in outdoor studies**. Aerosol Sci Technol. 54:520-546. DOI: 10.1080/02786826.2019.1676395

Theofel CG, Williams TR, Gutierrez E, Davidson GR, Jay-Russell M, Harris LJ. 2020. **Microorganisms move a short distance into an almond orchard from an adjacent upwind poultry operation**. Appl Environ Microbiol 86:e00573-20. https://doi.org/10.1128/AEM.00573-20.

Varma JK, Greene KD, Reller ME, et al. 2003. **An outbreak of *Escherichia coli* O157 infection following exposure to a contaminated building**. *JAMA*. 290(20):2709–2712. doi:10.1001/jama.290.20.2709

Verma M. 2021. **Field evaluation of microfluidic paper-based analytical devices for microbial source tracking.** CPS Funded Research